Please enter the following amendments to the specification shown below as clean paragraphs. A marked-up copy of each amended paragraph of the specification is attached to this amendment. Inserted material is indicated by underlining and deleted material is indicated by square brackets.

Clean copy of Page 3, Paragraph on Lines 14-24

A2

Each antibody comprises a glycoprotein molecule. The portion of an antibody molecule embodying the characteristic of shape or molecular topography, or code sequence which enables it to bind and so for example neutralise the antigenic determinant or epitope of an antigen is known as a "paratope". The paratope is conceptually a molecular region of a shape complementary to the epitope or to a part of the epitope of the antigen and is thought to reside in the so called hypervariable region of the antibody glycoprotein molecule.

Clean copy of Page 6, Paragraph on Lines 10-12

A3

According to one aspect the present invention consists of a method of treating an animal comprising the steps of:

Clean copy of Page 7, Paragraph on Lines 10-13

A4

According to a second aspect the present invention consists of a method of manufacture of an anti-paratopic antibody efficacious against mammalian infections comprising the steps of:

Clean copy of Page 8, Paragraph on Lines 23-26

A5

The antibodies, or paratypic paratope bearing segments of them, are utilized as an immunogen in a mouse host to produce mouse antibodies having anti-paratope characteristics.

Clean copy of Page 9, Paragraph on Lines 20-21

A6

Figure 5 (II) illustrates a general procedure for the purification of HIV antigen specific human antibodies.

Clean copy of Page 9, Paragraph on Lines 25-27

Figure 6a illustrates purification of human IgG prior to delineation into HIV/HIV antigen specific antibodies.

Clean copy of Page 10, Paragraph on Lines 1-3

Figure 6b illustrates purification of human IgA prior to delineation into HIV/HIV antigen specific antibodies.

A6 cont'd

Clean copy of Page 10, Paragraph on Lines 4-6

Figure 6c illustrates purification of human IgM prior to delineation into HIV/HIV antigen specific antigens.

Clean copy of Page 10, Paragraph Beginning on Line 27, through Page 11, Line 22

A7

Secondly, the present invention has as its basic premise the observation that epitope presentation within a particular species is unique to that species. The prior art technology looks to immuno-dominant epitopes common to all species and aims to produce mirror images to them. Epitopes selected in the prior art technology are identified using empirical approaches and numerous algorithms have been used to predict antigenic sequences. In the present invention, antigenic sequences necessary to produce neutralizing epitopes are believed to be both linear and assembled. Antigen presentation is a multifactorial operation involving several host immune components. Hence, the basic premise of the present invention is that epitope mapping algorithms while applicable do not identify all epitopes of immunological significance. It is in this area which the present invention is focused. The host immune system has a role in the amplification and display of the total repertory of epitopes of the invading immunogen. The present invention capitalises on this factor whilst prior art technologies have tended to approach the problem from a more conventional anti-idiotypic approach.

Clean copy of Page 12, Paragraph Beginning on Line 8, through Page 13, Line 4

A8

Finally, both the present invention and anti-idiotypic technology use hybridoma technology, protein chemistry and immunology. When testing the putative vaccine, however, anti-idiotype vaccines have to be tested for complementarity and efficacy in several animal species e.g. rabbits, sheep, baboons or chimpanzees etc. This is necessary to compensate for the interspecies approach used to generate and test the vaccine candidate. This is a relatively long and time consuming step.

The vaccine candidate produced in the present invention, however, has to be tested primarily for complementarity within the species to be immunized. It is designed to be primarily an intra-species approach. Accordingly, from a small pool of infected individuals either immune to a particular infectious agent or carrying neutralizing antibodies to it and using standard techniques (or minor technological variants) to produce anti-paratopic antibodies, it is possible using the present invention to generate specific vaccines against said infectious agent. These vaccines can then be used to treat a small number of infected individuals or to immunize an entire population of individuals prone to infection by said infectious agent.

A8 cont'd

Clean copy of Page 13, Paragraph on Lines 5-21

The particular advantage of the present invention is that in the case of some viruses, for example AIDS, there are so many epitopes some of which are protective, some of which are suppressive, some of which are dominant and some which have no effect on the immune system. In the present invention, selection of the antibody is not dependent on the epitope. Instead, selection is based on whether the antibody produced is neutralizing or not. In contrast, in prior art approaches, antibody selection depends on an epitope being common to a variety of different species. For example, if an antibody works in rabbits but not in guinea pigs, it is discarded. The result being to reject it but in doing so, the very epitopes which could protect human populations may be lost. This is overcome by the present invention because reliance is not placed on epitope recognition between species.

Clean copy of Page 14, Paragraph on Lines 13-20

A9

In the example under consideration the first stage is to select from the pool of human antibodies a prototypic set, in this case a set of immunoglobulins which effectively bind the aetiologic agent for Acquired Immune Deficiency Syndrome (AIDS). The generally accepted aetiological agent for AIDS is currently known as Human Immunodeficiency Virus hereinafter referred to as HIV.

Clean copy of Page 15, Paragraph on Lines 3-4

A10

If desired the retained immunoglobulin members so selected may be purified and used directly in step (3).

Clean copy of Page 17, Paragraph on Lines 9-13

A11

The human IgG/A/M is drawn from three main groups affected by AIDS viral infection, viz

- male homosexuals
- bisexual/female/heterosexual AIDS carriers
- hemophiliacs

Clean copy of Page 18, Paragraph on Lines 10-21

A12

Alternatively, the Ig sub-classes may be screened to select antigen specific antibodies for use as the immunogen. In this case, the Ig sub-classes are next screened for effectiveness against HIV antigen to select the most effective sub-classes in binding the antigen. More preferably, the antigen is first divided into sub-classes known as p18, p24, gp41, p55, gp120 and gp160. These antigen sub-classes differ from each other in molecular structure and can be separated by SDS-polyacrylamide gel electrophoresis. Each Ig sub-class is then screened against each antigen sub-class to select the most effective Ig's.

Clean copy of Page 19, Paragraph on Line 1

A13

Table 2

HTLV III - HUMAN SERUM Ig PARATOPE GRID

Clean copy of Page 20, Paragraph Beginning on Line 25, through Page 21, Line 6

A14

Human antibodies are excellent immunogens when injected into mice. The antigenic sites on the human antibody molecules are spread right across the length of the molecule from the NH_2 terminus-ie the Fab end to the carboxylic acid terminus -ie the Fc end. The Fab NH_2 end carries the paratope. Other antigenic components of the Fab are present for structural or "carrier" purposes. For the purposes of the vaccine the Fc exclusively exhibits "carrier" as opposed to paratope antigens.

Clean copy of Page 21, Paragraph Beginning on Line 7, through Page 22, Line 6

A'4 cont'd

Immunization studies have demonstrated that not all the antigenic sites on the intact human immunoglobulin molecule are of equal value in that a greater proportion of induced antibodies tend to be directed against the Fc region. This phenomenon is described as antigenic competition or more accurately as intramolecular antigenic competition. When developing an anti-paratopic antibody, however, the part of the molecule of most interest is the Fab area that is to say the paratope bearing region. A simple way to overcome the problem of Fc dominance is to enzymatically cleave the immunoglobulin molecule and isolate the Fab fragment. When used to immunize a mouse this will cause all the induced immunoglobulins to be directed against the Fab fragment. A subset of the anti-Fab antibodies generated by the mouse, irrespective of whether an intact immunoglobulin molecule or a Fab/F(ab)'2 fragment has been used, will be directed against the internal idiotope i.e. paratopic image of the human immunogen. Thus, the member of the anti-HIV prototypic set used as an immunogen in the mouse may be either (a) the mixed intact human immunoglobulin specific for the AIDS virus, (b) selected classes or sub-classes of the intact immunoglobulin, (c) a Fab/F(ab)'2 fragment of one or a combination of the AIDS specific immunoglobulins or (d) a Fab/F(ab)'2 fragment of one or a combination of the AIDS specific immunoglobulins complexed to carriers eg. Keyhole Limpet Haemocyanin or human albumin.

Cl.

Clean copy of Page ~~33~~ 23, Paragraph on Lines 24-27

A'5

(ii) given the diversity of the immunoglobulin response the antibody range may not be restricted and a more general immunization routine adopted. In the latter case

Clean copy of Page 24, Paragraph on Lines 7-9

A'6

While stating a preference for (i) an outline of the various alternative pathways for the purification and preparation of the immunogen is shown in Figs. 5 and 6A-C.

Clean copy of Page 30, Paragraph on Lines 9-12

A17

Sub genomic clones of HIV cDNA encoding gp120, gp41, p24 and p18 were cloned and amplified in *E. coli* using λ gt11. The *E. coli* lysates were screened with in-house and by commercial HIV antigen ELISA's.

Clean copy of Page 30, Paragraph on Lines 17-26

A18

Following precipitation of *E. coli* antigens with $(NH_4)_2SO_4$ the supernate was concentrated (Amicon) dialysed against distilled water and then against 0.05M Phosphate buffer pH7.2 (16hrs, 4°C). 40 mls of the dialysed concentrate was combined with approximately 2 ml of the IgG-Sepharose and the mixture incubated end-over-end for 2 hrs (RT). The matrix was exhaustively washed and the recombinant protein eluted using 4M $MgCl_2$, pH 8.3. The presence of recombinant antigen was confirmed as outlined above.

Clean copy of Page 32, Paragraph on Lines 14-25

A19

Mouse Ab2 antibodies were induced in the following way. 1.3×10^8 mouse spleen cells were recovered and washed in the incubation medium (Iscoves DMEM medium containing 20% foetal calf serum (FCS), 40% thymus conditioned medium (TCM), 5×10^{-4} M 2-mercaptoethanol, 4 mM L-glutamine 50 IU Penicillin and 50 IU streptomycin). HIV specific human immunoglobulins at a concentration of 10 micrograms/ml incubation medium was added to the mouse spleen cells. The total volume used in the incubation of the spleen cells with human antibody varied between 10 and 15 mls. In this example, the incubation was allowed to proceed for 7 days in a heated (37°C) CO_2 incubator.

Clean copy of Page 32, Paragraph Beginning on Line 27, through Page 33, Line 10

Following incubation the cells were recovered for fusion to either SP2, NS1 or X63-Ag*.653 mouse myeloma cells. The viability of the spleen cells was found to vary between 70 and 99% and the viability of the myeloma was generally 99%. For the sake of illustration SP 2 mouse spleen cells were used though other cells such as rat or human myeloma cells could be used in this procedure. Spleen cells were fused to the myeloma cells using polyethylene glycol 1500/4000

A19 cncld

(Boehringer/Mannheim) using standard procedures and following 24 hrs incubation in a CO_2 incubator at 37°C the hybrids were plated out in the incubation medium now containing HAT.

Clean copy of Page 34, Paragraph on Lines 11-14

Human peripheral blood lymphocytes (PBL's) were diluted 1:1 in phosphate buffered saline and the red cells removed by centrifugation through a Ficoll-hypaque cushion (Pharmacia).

A20

Clean copy of Page 34, Paragraph Beginning on Line 15, through Page 35, Line 2

The PBL's either depleted or not depleted of monocytes and lymphocytes using methods familiar to those skilled in the art, were then transformed using for example the EBV isolate B95-8 in sterile tissue culture media (RPMI-1640 + 5% FCS). In a simple example the B95-8 isolate is made available as a supernate which is mixed with the monocyte/T cell depleted fraction enriched for the B lymphocytes. The cells are grown in this mixture, fed as required, and expanded in 96-well flat bottomed plates prior to fusion with the mouse myeloma cell line such as X63-Ag*.653. Screening is by a commercially available HIV antibody ELISA. Cloning and feeding (Medium containing HAT/HT) is by the usual method except that non transformed will be selected out by feeding with 1 micromolar Oubain.